IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Takamasa WATANABE et al.

Serial No.: 10/568,761 Group Art Unit: 1644

Filed: February 21, 2006 Examiner: Haddad, Maher M

For: PREVENTION OR REMEDY FOR INFLAMMATORY BOWEL DISEASES CONTAINING ANTI-CD81 ANTIBODY AS THE ACTIVE INGREDIENT

DECLARATION UNDER RULE 1.132

Honorable Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Takamasa WATANABE, a citizen of Japan and residing at c/o Dainippon Sumitomo Pharma Co., Ltd. 1-98, Kasugadenaka 3-chome, Konohana-ku, Osaka-shi, Osaka 554-0022 Japan, say and declare as follows:

1. I was graduated from Faculty of Pharmaceutical Sciences, Hokkaido University, Japan, in 1989 and received a Master's degree in pharmaceutics at Hokkaido University in 1991.

2. My professional background is as follows:

1991-1994, I was an employee of Sumitomo Chemical Co., Ltd. and engaged in research and development works for purification process for antibody medicine.

1994-2005, I was an employee of Sumitomo Pharmaceuticals Co., Ltd and engaged in research and development of drugs for the treatment of following conditions/diseases:

1994-1999: immunological disease

2000-2005 : inflammatory bowel disease

2005-present, I have been an employee of Dainippon Sumitomo Pharma Co., Ltd and have been engaged in the research and development of drugs for treating inflammatory bowel disease.

3. I am an author or co-author of the following papers:

Experientia 48:371-374, 1992

Biochemistry International 27(1):1-8, 1992

Eur. J. Immunol. 29:413-418, 1999

4. I am one of the co-inventors of the above-identified application and am familiar with the subject matter thereof.

5. I have read the Office Action issued on June 16, 2008 and the reference cited therein and am familiar with the subject matter thereof.

6. In order to show that anti-CD81 antibodies including 2F7, Eat1 and Eat2 are effective for the treatment or improvement of IBD, the following experiment has been done under my direction.

Pharmacological effects of anti-CD81 antibodies to TNBS (2,4,6-trinitrobenzenesulfonic acid)-induced mouse colitis models

(1) Preparation of TNP-OVA

0.5 g of egg albumin (OVA: manufactured by Sigma) and 0.5 g of K_2CO_3 (manufactured by Nacalai Tesque) were dissolved in 25 ml of distilled water for injection (OVA solution). 0.5 g of 2,4,6-trinitrobenzenesulfonic acid (TNBS: manufactured by Nacalai Tesque) was dissolved in 25 ml of 0.1 M K_2CO_3 (TNBS solution). The OVA solution and the TNBS solution were mixed, and the mixture was stirred overnight at room temperature. The stirred solution was dialyzed against 0.01M $NaHCO_3$ (manufactured by Nacalai Tesque) through a dialyzer (Spectra/Por Membrane MWCO:10,000, manufactured by Spectrum Medical Industries Inc.). The dialyzate was subjected to protein quantitative determination by BCA Protein Assay Reagent (manufactured by Pierce).

(2) Division, sensitization, challenge and administration

Animals: 5-week-old, male, SJL/J mice (SJL/JOrlCrlCrlj) were used (supplied by Nippon Charles River).

A 1:1 emulsion of complete Freund's adjuvant (CFA: manufactured by Difco Laboratories) and 2 mg/ml of TNP-OVA was subcutaneously injected (sensitized) into the back of a mouse at a rate of 0.1 ml/head. On day 7 after the sensitization, 10 mg/ml of a TNBS 50% ethanol solution was intrarectally administered (0.2 ml/head was injected (challenged) by inserting a probe in a portion of up to 3 cm from the anus) under ether anesthesia. On day 6 after the challenge, the mice were divided

into five groups each consisting of 8 mice according to the weight and the score of the symptom. The groups are as follows:

a group of administering 0.2 mg (0.2 ml)/head of an anti-CD81 antibody solution (Clone: 2F7, manufactured by SouthernBiotech),

a group of administering 0.2 mg (0.2 ml)/head of an anti-CD81 antibody solution (Clone: Eat1, manufactured by Santa Cruz),

a group of administering 0.4 mg (0.2 ml)/head of an anti-CD81 antibody solution (Clone: Eat2, manufactured by BioLegend),

a pathogenic state control group of administering 0.4mg (0.2 ml)/head of a control hamster IgG solution (manufactured by Affinity BioReagents), and

a group of administering 200 mg/kg of sulfasalazine (SSZ: manufactured by Sigma) suspended in a 0.5% methylcellulose (manufactured by Nacalai Tesque).

In addition to the above 5 groups, 8 mice without receiving sensitization and challenge served as a non-pathogenic state control group.

To the antibody administering groups and the pathogenic state control group, the antibody was intraperitoneally administered on the division day. To the SSZ administering group, SSZ was orally administered continuously once daily from the division day. On day 7 after the division, the experiment was completed.

(3) Evaluation with symptom score

The symptom of colitis from the division day to day 7 after the division was observed. The symptom of colitis was scored according to the conditions of stool [normal stool (score:0),

loose stool (score:1) and diarrhea (score:2)]. Dead individuals were excluded from the results. With respect to the statistic treatment, round-robin multi-group comparison of the symptom of colitis on the day 7 after the division, the pathogenic state control group, the anti-CD81 antibody administration group, the SSZ administration group and the non-pathogenic state control group was conducted by the Steel test (*: $0.01 < p < 0.05$, **: $p < 0.01$).

(4) Measurement of a length of a large bowel

On the final day of the experiment, after euthanasia with a high concentration of CO_2, a large bowel was extracted, and the length thereof was measured. Regarding the length of the large bowel in each group, round-robin statistic treatment in the pathogenic state control group, the anti-CD81 antibody group, the SSZ administration group and the non-pathogenic state control group was conducted by a Dunnet test after a Bartlett test (*: $0.01 < p < 0.05$, **: $p < 0.01$).

The results were shown in Table 1.

Table 1

Group	Symptom of colitis (SEM)	Intestinal Length mm(SEM)
Non pathogenic state control	0.00(0.00)**	110.5(1.7)**
Pathogenic state control	1.88(0.13)	91.1(2.4)
Anti-CD81 antibody (2F7)	0.5(0.27)*	103.5(1.4)**
Anti-CD81 antibody (Eat1)	0.75(0.31)*	104.5(0.9)**
Anti-CD81 antibody (Eat2)	0.63(0.32)*	105.8(2.3)**
SSZ administration	0.63 (0.18)**	104.9 (2.0)**

As shown in Table 1, all of the three anti-CD81 antibodies

tested were effective for the treatment of symptom of colitis of the TNBS-induced colitis mouse and for improving the shortening of the intestinal length.

7. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

This 13th day of November 2008

Takamasa Watanabe

Takamasa WATANABE